A STUDY OF THE EXTERNAL BREATHING-APPARATUS OF THE LARVÆ OF SOME MUSCOID FLIES.

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(Plate lxx.)

Heretofore, in the identification of different species of flies from maggots collected, the perfect flies have had to be bred-out. But, in the course of the work of the Government Sheep-Fly Experiment Station, this method was often impossible, when packets of maggots were received dead or rotten. By means of the microscope, I have found that an examination of the spiracles, in particular the posterior spiracles, gives a sure and certain means of identifying the different species here dealt with. As their description has not been published heretofore, the results are here recorded. In every case, the types were procured from eggs deposited by each species of fly, on meat in the field-laboratory.

The species dealt with in this paper are:—

Anastellorhina augur (*Calliphora oceaniæ*).
Pollenia stygia (*Calliphora villosa*).
Pycnosoma rufifacies (*Calliphora rufifacies*).
Pycnosoma varipes (*Calliphora varipes*).
Lucilia sericata.
Ophyra nigra.

The spiracles, in all probability, exercise a most important function in the destruction of maggots by poison. Careful examination of the skin does not reveal the presence of any pores; the organs of the maggot may be looked upon as being enclosed in an india-rubber-bag, the only openings into which are the gullet and anus—the extremities of the alimentary tract—and the anterior and posterior spiracles—the extremities of the respiratory system. These anterior and posterior spiracles are connected by two main tracheæ, from which branches ramify

throughout the body. Absorption will, in all probability, take place slowly through the skin; but the action of some liquids is far too rapid to admit of this happening, so that, in these cases, attention must be paid to the openings into the body. I am not at present in a position to point out more than the possibilities of this point, but it is receiving further attention.

The absence of pores in the skin of the maggots of these species is extremely interesting as well as curious, because the larvæ of most other insects, and even diptera, show definite, well-marked pores.

The anterior and posterior spiracles are totally different from one another, the most marked specific differences being given by the latter.

The anterior spiracles are situated one on either side of the body, on the first thoracic segment. In shape, they resemble a cap with a sharp upper edge, and a row of tassels along this edge. The caps are chitinised projections of the two main tracheæ on which they fit by a thickened ring; and it is from this demarcation-line that all measurements are taken. In some species, the cap is differentiated into a thicker lower part, and a thinner upper part. The tassels or tubules are hollow, the open ends having a much-strengthened chitinous rim to keep the apertures open. The number of tubules varies in different species, and within certain limits in the same species, and even between the right and left anterior spiracles in the same maggot.

The tracheæ terminate posteriorly on the anal segment in two spiracular plates or stigmata, the posterior spiracles. These are slightly raised above the surface of the truncated end of the maggot. The position of these plates on the body is such that the bases of the plate, *i.e.*, the spaces in the band, point obliquely towards the median perpendicular plane of the body, and with the median horizontal plane of the body passing through the lower portion of the plates.

As both spiracular plates are alike for each species, a general description of the characters of one will serve equally for the other.

The plate is surrounded by a strong band of chitin, varying in thickness and in outline in different species. At the base is a space or opening in the band; in *Ophyra nigra*, however, this is closed up; and, in *Pycnosoma rufifacies* and *P. varipes*, it is open, forming a break in the band. This space appears to carry some soft structure in life, but which is destroyed in preparation. Within this band are three structures radiating slightly from the base; on subjection to strain, it is seen that these are slits, the openings being crossed by bands of chitin, either straight or anastomosing. The side-walls of the slits are very much strengthened with chitinous blocks, and the ends are always rounded. They are also concave from side to side.

In some species here dealt with, there is an intermediate structure lying between the middle and inner slits—"inner" referring to the side nearest the median line of the body. This is a shallow groove, somewhat V-shaped, coming to a more or less definite point towards the bottom, and rounded at the top. The rounded end encloses a clear space, the margin of which shows, under a low magnification, a "sun-ray" effect. This is due to fine bands of chitin radiating outwardly to the band. The inner ends of these chitinous bands project over the rim of the clear space, and would seem to support some soft structure in life, but which is destroyed by the preparation. In some species, also, a space resembling a blister occurs on the side of the slits; when present, it is always on the outer side of the outer and middle slits, and on the inner side of the inner slit. This "blister-structure" also shows the "sun-ray" effect referred to in the "intermediate-structure."

The length and breadth of all the spiracular plates include the width of the band.

I am indebted to Professor Wheeler, of Harvard University, U.S.A., for the method of preliminary preparation of the maggots. Full-grown maggots are dropped into water heated to 80°C. A few seconds in water at this temperature is sufficient to kill them, and has the great advantage of stretching the maggots to their full length; by killing in alcohol, the maggots generally

contract, and often curve the body. The maggots are then placed in 35% alcohol, and, after about 14 hours, they are transferred to 75% alcohol, in which they can be preserved. As a general rule, the maggots are left perfectly white by this preparation, excepting occasionally when they have been feeding on material rich in blood. When ready for preparation as microscopic specimens, the body of the maggot is slit along the median basal line, and boiled in 10% KOH until only the integument remains. This is then put through the usual process for such preparations, and mounted in Canada Balsam. All measurements are in fractions of a millimètre.

The bibliography on the spiracles of dipterous larvæ is, so far as I have been enabled to trace it, very scanty. Figures of the spiracles of different species have been given on a number of occasions; but when a description is given, it is brief and without detail.

Nielsen(1) figures the spiracles of certain species of *Tabanidæ*, but goes no further.

Froggatt(2) figures the spiracle of a dipterous larva found parasitic in locusts at Minimbah Station, near Singleton, N.S.W. A curious feature in this species was an elongated, cone-shaped tail fitting over the anal portion of the abdomen. This was doubtless to protect the spiracles, and prevent the maggot from being smothered when the legs and wings were folded. The perfect fly was never bred out, so that it could not be identified.

Gurney(3) figures the spiracles of three species of fruit-flies, and states that the identification of the different species can be thus determined, but gives no description.

Banks(4) gives a slightly more extended account of the spiracles of the different species he deals with, but gives very little detail. He refers to what I am calling the space in the band of the anal spiracular plate, as a "button"; in which I do not agree with him. He does not say how the spiracles were examined.

As extreme forms of spiracles, the following may be given. *Eristalis tenax*, "The Common Drone-Fly," an introduced species common in gardens, is the parent of curious rat-tailed larvæ which can live not only in putrid but even in salt water. The

posterior spiracles are projected into a long tail in order to enable it to breathe while completely submerged.

The larvæ of *Stratiomya chamæleo*, another European fly which swarms in water, have a similar, elongated, tail-like projection of the spiracles. The larvæ of a Tachinid fly (*Gymnosoma rotundatum*) live in and feed on the body of a Shield-Bug, and, in order to breathe, have an elongated, hook-like projection of the spiracles, which is pushed through one of the spiracles in the side of the body of the bug.

ANASTELLORHINA AUGUR. (Plate lxx., figs.4, 4*a*).

Anterior Spiracles.—Cap differentiated into two parts. Tubules very strong.

Posterior Spiracles.—The band strong, complete, not markedly thick, showing no differentiation into two parts as in *P. rufifacies* and *P. varipes*. The band is thickened round the space which projects the contour outwards at this point. The band is drawn in slightly between the slits, giving the contour a slightly scalloped appearance. The inner margin of the band projects very slightly between the outer and middle slits, and less markedly between the middle and inner slits. The slits are fairly close together at the base, but are well separated otherwise, and do not run quite the full length of the plate. The outer and inner slits are fairly straight throughout their whole lengths. The middle slit is straight for about two-thirds of its length, and then inclines inwards. "Intermediate structure" always present between the middle and inner slits. "Blister-structure" well marked on all three slits.

POLLENIA STYGIA. (Plate lxx., figs.6, 6*a*).

Anterior Spiracles.—Cap differentiated into two parts. Tubules broad compared with their length, and strong.

Posterior Spiracles.—Band strong and complete. Space at base completely enclosed in the band, which is not projected outward to such an extent as in *A. augur*. The band is only slightly drawn inwards between the slits. The inner margin of the band projects strongly downwards between the middle and

outer slits, and somewhat less strongly between the middle and inner slits; in the latter case, this projection usually forms a hollow above the space in the upper end of the "intermediate structure." Slits fairly close together, and running practically the whole length of the plate. Outer and inner slits fairly straight throughout their whole lengths. The lower half of the middle slit is fairly straight, from which point it bends slightly inwards. "Blister-structure" usually situated just above the point of curvature. "Intermediate structure" always present between the middle and inner slits, and well developed. In a few cases, a similar structure has been noticed between the outer and middle slits, but then the "blister-structure" on the middle slit is absent. "Blister-structure" well developed on the slits. The "sun-ray" effect is particularly well marked on the "blister-structures" and on the space in the top of the "intermediate structure."

PYCNOSOMA RUFIFACIES. (Plate lxx., figs.5, 5*a*).

Anterior Spiracles.—Cap differentiated into two parts. Tubules long.

Posterior Spiracles.—Band very strong, often differentiated into two parts, the chitinous material being thinner on the outer portion. It would seem that, when the maggots are young, the differentiation in the band is most marked, but as development is carried on, this differentiation decreases until the two parts become either fused or the outer becomes the thicker and stronger. There is, of course, no space between these two parts in the band. The band is broken at the base, leaving the space open and incompletely surrounded. The band is always much thicker at the base. The inner edge of the band projects downwards slightly between the middle and inner slits, and less markedly between the middle and outer slits. The outer margin of the band is regular in outline. The slits run the full length of the plate, abutting on the inner edge of the band at the top and bottom of the spiracular plate. The outer slit, in shape, is fairly straight on the inner side, the outer side being slightly convex, following the outline of the inner margin of the band. The lower part of the middle slit is fairly straight, and close against the

base of the outer slit. About the middle of its length, it bends inwards slightly. "Blister-structure," when present, generally situated about the point where the inward bend takes place. The outer edge of the inner slit is fairly straight; the inner edge follows more the contour of the inner margin of the band. It is slightly convex. "Blister-structure" at approximately the maximum point of curvature. The "intermediate structure" occurs between the middle and inner slits, but, in many cases, it is but slightly developed. The "blister-structure" is generally present on the inner side of the inner slit, and absent on the other two slits. It may, however, be seen on all three slits.

Pycnosoma varipes. (Plate lxx., figs.3, 3*a*).

Anterior Spiracles. Cap differentiated into two parts. Tubules much shorter than in *P. rufifacies.*

Posterior Spiracles.—Band extremely thick and strong, and showing the same differentiation into two parts as in *P. rufifacies;* the same remarks apply equally in this case as in the previous one. The inner margin of the band projects slightly downwards between the outer and middle slits. The band is broken at the base, leaving the space open and incompletely surrounded. On the inner side of the spiracular plate, the band shows a development of chitin like a square flag—the minimum distance between the plates has been taken from the edges of these flags. The slits run the whole length of the plate. The outer slit is fairly straight and regular throughout its whole length, although the outer margin conforms more or less to the contour of the inner margin of the band. The "intermediate structure" is only very slightly developed between the middle and inner slits, and is apparently only noticeable in the later stages of the growth of the maggot. The "blister-structure" on the slits is absent.

Lucilia sericata. (Plate lxx., figs.2, 2*a*).

Anterior Spiracles.—Cap not differentiated into two parts. Tubules short and slender.

Posterior Spiracles.—Band fairly thin and entire, pinched in between the slits as in *A. augur.* The space in the band is completely enclosed by the band, which is thickened at this spot;

the space generally tends to project the inner side of the band inwards. There is no projection of the inner margin of the band between the slits. The slits radiate out from the base, and fill the greater part of the spiracular plate, but do not run the full length of it. In shape, the slits are all very similar, being, as a rule, fairly straight and regular. The "intermediate structure" is well developed, and always present between the middle and inner slits. The "blister-structure" is always present on the slits, and well developed. The blister seems, more noticeably in this species, to be contained in a sheath which generally runs a long way along the slit on either side of the blister; this is generally more pronounced on the middle slit.

OPHYRA NIGRA. (Plate lxx., figs. 1, 1*a*).

Anterior Spiracles.— Cap not divided into two parts. Tubules long and slender.

Posterior Spiracles.— Band complete, and comparatively thick and strong. It is differentiated, in the earlier stages of the development of the maggot, into two parts, the inner part being the broader and stronger. It is regular in outline. The space is not in the band, but is surrounded by a development of chitin at the base of the plate, resting against the band. The space is almost completely filled by a development of chitin. The bases of the inner and outer slits rest against this ingrowth of chitin. Compared with the size of the spiracular plate, the slits are small, and appear to be well separated from one another. This is due to each slit being surrounded by an envelope of hyaline material, which envelopes are, however, lying up against one another. The inner margin of the band does not, as a rule, project downwards at all between the slits The outer and inner slits are usually fairly straight and regular to almost the upper end, where they bend over slightly outwards and inwards respectively. They may, however, present a slightly wavy appearance. The middle slit is always wavy in outline, with the tip of the upper end bending slightly over. "Intermediate" and "blister-structures" are absent.

		O. nigra.	*L. sericata.*	*P. varipes.*	*P. rufifacies.*	*Pol. stygia.*	*A. augur.*
		mm.	mm.	mm.	mm.	mm.	mm.
Anterior Spiracles	Cap: Total length	0·1640	0·1312	0·1192	0·1558	0·1517	0·1394
	Cap: Length of upper part	—	—	0·0441	0·0820	0·0502	0·0583
	Cap: Length of lower part	—	—	0·0747	0·0738	0·1015	0·0811
	Cap: Breadth of upper part	0·1550	0·1230	0·1107	0·1292	0·2306	0·1609
	Cap: Breadth of lower part	0·1343	—	0·1025	0·1640	0·2152	0·1785
	Tubules: Number	7 to 9	8 to 9	9 to 10	10 to 11	10 to 13	9 to 11
	Tubules: Length	0·0410	0·0246	0·0287	0·0492	0·0328	0·0328
	Tubules: Breadth	0·0082	0·0082	0·0139	0·0164	0·0164	0·0164
Posterior Spiracles.	Min. distance between spiracular plates	0·1099	0·1743	0·0656	0·1640	0·1991	0·2583
	Length of spiracular plate	0·1740	0·2109	0·2403	0·3835	0·4152	0·2821
	Breadth of spiracular plate	0·1831	0·2738	0·2433	0·3617	0·4177	0·2852
	Length of intermediate structure	—	0·0820	—	0·1722	0·2112	0·1140
	Max. dist. between outer & middle slits	—	0·0389	0·0164	0·0338	0·0864	0·0629
	Min. dist. between outer & middle slits	—	0·0082	Rt. together	0·0072	0·0126	0·0082
	Max. dist. between middle & inner slits	—	0·0375	0·0246	0·0370	0·0840	0·0528
	Min. dist. between middle & inner slits	—	0·0164	Rt. together	0·0154	0·0240	0·0284
	Length of outer slit	0·0767	0·1312	0·1312	0·2378	0·2964	0·1817
	Breadth of outer slit	0·0246	0·0410	0·0401	0·0681	0·0678	0·0410
	Dist. from base of "blister-structure"	—	0·0292	—	0·0793	0·1135	0·0656
	Length of "blister-structure"	—	0·0574	—	0·0793	0·0880	0·0574
	Length of middle slit	0·0845	0·1394	0·1509	0·2533	0·3216	0·1948
	Breadth of middle slit	0·0246	0·0400	0·1410	0·0647	0·0691	0·0410
	Dist. from base of "blister-structure"	—	0·0656	—	0·1585	0·1838	0·1239
	Length of "blister structure"	—	0·0445	—	0·0519	0·0689	0·0389
	Length of inner slit	0·0877	0·1343	0·1369	0·2232	0·2941	0·1886
	Breadth of inner slit	0·0246	0·0410	0·3936	0·0665	0·0715	0·0410
	Dist. from base of "blister-structure"	—	0·0293	—	0·0902	0·1335	0·0948
	Length of "blister-structure"	—	0·0691	—	0·0683	0·0770	0·0328

BIBLIOGRAPHY.

(1) Nielsen, J. C.—"Om Gymnosoma. Arteners Biologi (Dipt. Tachin). Undersogelser over entoparasitiske Muscidelarver hos Arthropoder vi.

(2) Froggatt, W. W.—"Eastern Plague-Locust." Agric. Gazette N. S. Wales, June, 1907.

(3) Gurney, W. B.—"Fruit-Flies at Narara." Agric. Gazette N. S. Wales, 1912.

(4) Banks, N.—"Structure of certain species of Dipterous Larvæ, with particular reference to those in human Foods." U.S. Department of Agriculture, Entom. Bureau, January, 1912.

EXPLANATION OF PLATE LXX.

Anterior and posterior spiracles of *Ophyra nigra* (Figs. 1, 1*a*)—*Lucilia sericata* (Figs. 2, 2*a*)—*Pycnosoma variipes* (Figs. 3, 3*a*)—*Anastellorhina augur* (Figs. 4, 4*a*)—*Pycnosoma rufifacies* (Figs. 5, 5*a*)—*Pollenia stygia* (Figs. 6, 6*a*).